IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Tsang et al.

Serial No.: 09/633,034 Examiner: Huff, Sheela Jitendra

Filed : August 4, 2000 Group Art Unit: 1643

For : MONOCLONAL ANTIBODIES AGAINST HUMAN COLON CARCINOMA-ASSOCIATED ANTIGENS AND USES THEREFOR

DECLARATION OF DR. JEFFREY FASICK UNDER 37 C.F.R. §1.132

I hereby certify that this paper is being deposited on May 9 ~~April 10, 2006~~ with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria VA 22313-1450

Lisa B. Kole
Attorney Name

Signature

35,225
PTO Registration No.

May 9 2006
Date of Signature

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Jeffrey Fasick, declare the following:

1. I am Director of Science of International Bioimmune Systems, Inc. ("IBS"), assignee of the above-identified application.

2. I hold a Ph.D. from the Department of Biological Sciences of the University of Maryland, and have a substantial research background in the field of molecular biology, as illustrated by my *Curriculum Vitae*, a copy of which is attached hereto (Exhibit 1).

3. This declaration relates to biological deposits made under the provisions of the Budapest Treaty of hybridomas designated PCA 31.1 and PCA 33.28 which produce monoclonal antibodies 31.1 and 33.28. Earlier deposits of the hybridomas were found to lack the expected immunospecificity. These hybridomas were therefore re-deposited.

4. The above mentioned hybridomas were re-deposited under the Budapest Treaty at the American Type Culture Collection (ATCC), Manassas, VA, with the following depository details. PCA 31.1 was re-deposited at the ATCC on September 22, 2000 and assigned the Patent Deposit Designation PTA-2497. As proof of deposit, a copy of the corresponding International Form is provided (Exhibit 2). PCA 33.28 was re-deposited at the ATCC on August 26, 2003 and assigned the Patent Deposit Designation PTA-5413. As proof of deposit, a copy of the corresponding International Form is provided (Exhibit 3).

5. In my previous declaration, submitted July 8, 2005, I had noted that neither murine mAb 31.1 nor its "humanized" chimeric version mAb react with proteins in the Hollinshead colon adenocarcinoma TAA vaccine samples in the IBS freezer. I had hypothesized that this might be due to either (i) degradation of the target antigen in the vaccine (which we deemed unlikely) or (ii) absence of target antigen in the vaccine preparation tested. I had stated, in paragraph 7 of my previous declaration, that the "fact that the Hollinshead TAA vaccine in the IBS freezer does not react with monoclonal antibodies covered in the '657 patent may be explained, in my opinion, by the fact that even though the protocols used to prepare immunogen were presumably similar, they were not necessarily the same; further, I believe that the tumor sources were not the same."

6. In this declaration, I report the results of subsequently performed experiments, performed by me or under my direction, which have repeated and confirmed the result reported in my previous declaration. The results of one such experiment are presented in Figure 1 (Exhibit 4). In a Western blot of cell lysates from ASPc1 (a human pancreatic cancer derived cell line) and LS147T (a human colon carcinoma derived cell

line), an approximately 40 kd band was detected by both chimeric 31.1 antibody (upper panel) and murine 31.1 antibody from PTA-2497 hybridoma (lower panel).

7. Chimeric 31.1 antibody is an accurate representation of the antigen specificity of the original murine 31.1 monoclonal antibody disclosed in the '657 patent because it is derived from that original murine 31.1 antibody. A cell line producing the chimeric version of 31.1 antibody was deposited with the American Type Culture Collection during the original prosecution of the '657 patent, where it was referred to as "Chi #1" and assigned Accession No. CRL 12316 (in the '657 patent specification, column 3 lines 65-67). The identity of that deposit was never questioned, and Chi#1 was *not* re-deposited. The recent experimental results presented in Figure 1 (Exhibit 4) show chimeric 31.1 reacting with colon cancer derived cell line LS174T; consistent with these results, the '657 patent discloses that Chi#1 stained LS174 in cytofluorometry studies and lysed LS174T cells in antibody-dependent cell-mediated cytotoxicity tests. Therefore there is no reason to doubt that the Chi#1 antibody used to generate Figure 1 (Exhibit 4) correctly represents the original murine 31.1 antibody specificity.

8. That the chimeric 31.1 and the re-deposited murine 31.1 antibodies both bind to the same size antigen in two different cancer cell lines demonstrates, in my opinion, that (i) the re-deposited murine 31.1 hybridoma is correct and represents the original disclosure of the '657 patent and (ii) the size of the target antigen, originally reported to be about 72 kilodaltons, was in error.

9. Further, experiments have now been performed to verify the correctness of the re-deposited 33.28 hybridoma. As seen in Figure 2 (Exhibit 5), an approximately 60-70 kd band was detected by antibody prepared form a sample of 33.28 hybridoma cells stored at IBS (upper panel) as well as 33.28 antibody produced by re-deposited hybridoma PTA-5413 (lower panel) in the lane containing cell lysates from WiDR cells (human colon carcinoma derived cell line). In my opinion, the observed antigen size in these recent experiments is consistent with the size disclosed in the '657 patent, 61.1 kd. This, together with the fact that antibody produced by both company-stored and re-deposited hybridomas reacted with the same size antigen, demonstrate to me that the re-deposit of 33.28 hybridoma cells is correct.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing from the above-captioned patent application.

Dated: May 7, 2006 By: Jeffrey D. Fasick
Jeffrey Basick

Jeffry I. Fasick, Ph.D.

6 Jefferson Street
Port Washington, New York 11050

Cellular Phone: 646-491-0171
Email: FASICK_IBS@YAHOO.COM

1. Employment History

2003-Present **Director of Science**
International Bioimmune Systems, Inc.

225 West Community Drive Great Neck, New York 11021
Duties: In Charge of All R&D Labs and Personnel; Responsible for Lead Product Development

2002-03 Senior Research Scientist

International Bioimmune Systems, Inc.
225 West Community Drive Great Neck, New York 11021
Research: Development of Immunotherapeutic mAbs Specific for Colon and Pancreatic Tumor Associated Antigens

1999-2002 **Scientific Consultant, Proctor and Gamble Pharmaceuticals**
G-Protein Coupled Receptor Group, Dr. Robert Barnett (Group Head)
Health Care Research Center
Proctor and Gamble Pharmaceuticals, Mason, Ohio 45040
Research: Cloning, Expression, and Purification of the Human Melanocortin-4 Receptor Gene

1998-2002 **Postdoctoral Fellow**
Laboratory of Professor Daniel D.Oprian (Department Chair)
Department of Biochemistry and the Volen Center for Complex Systems
Brandeis University Waltham, Massachusetts 02454
Research: Molecular Mechanisms of Spectral Tuning in Mammalian Photoreceptor Visual Pigments

2. Education

1993-98 **Ph.D.**
Department of Biological Sciences
University of Maryland Baltimore County
Baltimore, Maryland 21250

1990-93 **M.S.**
Department of Biological Sciences
University of Maryland Baltimore County
Baltimore, Maryland 21250

1984-88 **B.S.**

Department of Ecology, Ethology, and Evolution
University of Illinois, Urbana-Champaign
Urbana, Illinois 61801

Experience / Accomplishments

Director of Science — **International Bioimmune Systems, Inc.**
June 2003-Present — **Great Neck, New York**

- Wrote Pre-IND briefing document for a Phase 1 clinical trial
- Represented sponsor and presented at Pre-IND meeting with the FDA
- Wrote the International Bioimmune Systems, Inc. Executive Summary Statement
- Produced the International Bioimmunce Systems, Inc. Research Summary Presentation
- Evaluated/contracted out-sourcing partners for GMP manufacturing, tissue cross-reactivity testing, and animal studies per FDA recommendations
- Presented data, research, and records to potential investors and collaborators
- Presented current research at annual shareholders meetings
- Liaison for Principle Investigators for Phase 1 Clinical Trials
- Designed/implemented S.O.P. protocols
- Designed/implemented cGMP-like manufacturing protocols for immunotherapeutic mAbs
    - High expression/high viability in stirred reactor
    - Multi-step purification with robust viral removal
    - Asceptic Fill/Finish
    - Quality Control Testing
- Initiated and supervised the immunopurification of tumor associated antigen for identification by MALDI MS
- Initiated and supervised the characterization of immunotherapeutic mAbs by tissue cross reactivity testing, cell flow cytometry, cell based ELISA assays, cellular cytotoxicity assays, animal models including pharmacokinetic and toxicity studies
- Reviewed and edited patent applications, reissues, supplements, and office actions in coordination with patent attorneys and agents
- Created alliances with contractors for all facility maintenance, repairs, and services
- Collaborations: D. Webb (Wellesley University), Oncovation (Brooklyn, NY)

Senior Research Scientist — **International Bioimmune Systems, Inc.**
June 2002-June 2003 — **Great Neck, New York**

- Developed cell line expressing immunotherapeutic mAb against colon/pancreatic cancer
    - Developed human-murine chimeric immunotherapeutic mAb
    - Developed stable expression immunotherapeutic mAb clones in CHO_{dhfr-} cells
    - Amplified immunotherapeutic mAb expression levels in CHO_{dhfr-} cells
    - Adapted mAb expressing CHO_{dhfr-} cells to suspension and serum-free media
    - Created master and working cell banks
    - Deposited cell lines and plasmids with ATCC
    - Applied for patent supplement
- Developed clonogenic assay for immunotherapeutic mAbs
- Acquired expertise in column chromatography for protein purification
    - Immunoaffinity
    - Ion Exchange
    - Gel Filtration
    - FPLC
- Worked closely with outsourcing partner on the manufacturing of immunotherapeutic mAb

- Cloned and expressed tumor associated antigen specific for immunotherapeutic mAb
- Characterized the glycosylation pattern of tumor associated antigen
- Collaborations: I. Wang (Medical University of South Carolina), K. Tsang (National Cancer Institute), J. Schubert (Cell Trends, Inc.)

Postdoctoral Fellow — **Brandeis University**
April 1998-June 2002 — **Waltham, Massachusetts**

- Developed large scale expression and purification system to study G-protein coupled receptors (GPCRs) from mammalian cells and suspension cultures
- Designed custom media utilized in N^{15} labeled protein expression system and expressed novel GPCRs for subsequent NMR analysis
- Performed site-directed mutagenesis to examine protein/ligand interactions and structural confirmations of GPCRs
- Performed G-protein and kinase binding assays to study protein activity
- Performed transgenic xenopus preparations for the expression of human GPCRs
- Performed DNA cloning, expression, and characterization of a variety of novel mammalian visual pigment genes
- Performed UV/visible light spectroscopy to analyze the spectral tuning properties of visual pigments
- Supervised graduate student rotation projects
- Collaborations: T. Smith (SUNY Stonybrook), M. Applebury (Harvard Medical School)

Research Fellow — **University of Maryland Baltimore County**
September 1993-March 1998 — **Baltimore, Maryland**

- Prepared and screened cDNA libraries
- Conducted large- and small-scale plasmid preparation in E. coli
- Utilized stable and transient transfection techniques in mammalian cell lines
- Designed and implemented protocols to secure fresh tissue from dead stranded marine mammals
- Designed technique to dissect and process fresh tissue for total RNA and genomic DNA extraction
- Acquired expertise in PCR and RT-PCR; Maxam-Gilbert and Sanger dideoxy DNA sequencing; Northern, Southern, and Western blot analysis techniques, restriction fragment analysis; autoradiography,
- Performed phylogenetic analyses using PHYLIP, CLUSTAL V, MEGA, GCG Wisconsin package, and PAUP*
- Performed DNA analysis using FASTA, BLAST, and DNA Strider
- Skilled in Powerpoint, EXCEL, Kaleidagraph, Adobe, Chemdraw, Rasmol, and WebLab Veiwer
- Acquired expertise in light and electron microscopy (SEM & TEM).
- Utilized the training of computer generated neural networks
- Acquired expertise in performing immunoaffinity chromatography
- Collaborations: National Aquarium in Baltimore, National Marine Fisheries, Chicago Zoological Assn., H. Howland (Cornell University), Virginia Marine Science Museum, Smithsonian Institute National Museum of Natural History, Maryland DNR

Additional Training

- Protein Chromatography and FPLC Seminar Series, October 11-15, 2004, GE Healthcare (Amersham Biosciences)
- Good Manufacturing Practices, February, 2004, Schiff & Company, West Caldwell, NJ

- Cell & Tissue Reactor Engineering. July 15-18, 2002. Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN.
- Cell Culture Techniques. June 25-28, 2002. The Biotechnical Institute of Maryland, Inc. University of Maryland-Baltimore. Baltimore, MD.
- Workshop on Transgenics. June 2-3, 1999. Biotechnology Center, University of Connecticut, Storrs, CT.
- Workshop on Molecular Evolution. August 3-15, 1997. Marine Biological Laboratory, Woods Hole, MA.

3. Publications

- Fasick JI, Applebury ML, Oprian DD. Spectral tuning in the mammalian short-wavelength sensitive cone pigments. *Biochemistry*. 2002 May 28;41(21):6860-5.
- Fasick, JI, Robinson PR. Cloning and expression of dolphin opsin sequences and a mechanism of sprectral tuning. Cell and Molecular Biology of Marine Mammals, Pfeiffer, C.J., Ed., Krieger Press, Melbourne, 2002.
- Fasick JI, Robinson PR. Spectral-tuning mechanisms of marine mammal rhodopsins and correlations with foraging depth. *Vis Neurosci*. 2000 Sep-Oct;17(5):781-8.
- Fasick JI, Lee N, Oprian DD. Spectral tuning in the human blue cone pigment. *Biochemistry*. 1999 Sep 7;38(36):11593-6.
- Cronin, TW, Fasick, JI, Howland, HC. Video photoretinoscopy of the eyes of the small odontocetes (*Tursiops truncates, Phocoena phocoena, and Kogia breviceps*). *Mar. Mamm. Science* 1998 14: 584 90.
- Fasick JI, Cronin TW, Hunt DM, Robinson PR. The visual pigments of the bottlenose dolphin (*Tursiops truncatus*). *Vis Neurosci*. 1998 Jul-Aug;15(4):643-51.
- Fasick JI, Robsinson PR. Mechanism of spectral tuning in the dolphin visual pigments. *Biochemistry*. 1998 Jan 13;37(2):433-8.

4. Published Abstracts

- Fasick, JI, Oprian, DD. 2001. Spectral tuning in the mammalian short-wavelength sensitive cone visual pigments. FASEB Biophysics 80: 601a.
- Fasick, JI, Robinson, PR. 1996. Molecular cloning and characterization of visual pigments in the bottlenose dolphin (*Tursiops truncates*). Society for Neuroscience 22: 792.16.

Departmental Seminars/ Invited Talks

- 10th International Conference on Retinal Proteins. August 20-24, 2002. University of Washington, Seattle, WA. Title: Spectral Tuning in the Mammalian SWS-1 Cone Pigments.
- Department of Zoology, University of New Hampshire, Durham, NH. March 1, 2002. Title: Spectral Tuning in the Mammalian Short-Wavelength Sensitive Cone Pigments.
- 25th Annual Meeting of IMATA. October 20-25, 1997. Baltimore, MD. Title: The Visual Pigments of the Bottlenose Dolphin (*Tursiops truncates*).

ATCC

10801 University Blvd • Manassas, VA 20110-2209 • Telephone: 703-365-2700 • FAX: 703-365-2745

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

International BioImmune Systems
Attn: Andrew Lin
225 W. Community Drive, Suite 140
Great Neck, NY 11021

Deposited on Behalf of: International BioImmune Systems

Identification Reference by Depositor:
Mouse hybridoma cell line: PCA 31.1-AT

Patent Deposit Designation
PTA-2497

The deposit was accompanied by: __ a scientific description __ a proposed taxonomic description indicated above.

The deposit was received September 22, 2000 by this International Depository Authority and has been accepted.

AT YOUR REQUEST: X We will inform you of requests for the strain for 30 years.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested October 9, 2000. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Tanya Nunnally, Patent Specialist, Patent Depository

Date: February 6, 2001

cc: Lisa B. Kole

ATCC

10801 University Blvd • Manassas, VA 20110-2209 • Telephone 703-365-2700 • FAX: 703-365-2745

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

International BioImmune Systems, Inc.
Attn: Dr. Jeffrey I. Fasick
235 West Community Drive, Suite 140
Great Neck, NY 11021

Deposited on Behalf of: International BioImmune Systems, Inc.

Identification Reference by Depositor:	Patent Deposit Designation
Mouse Hybridoma: PCA 33.28	PTA-5413

The deposit was accompanied by: __ a scientific description _ a proposed taxonomic description indicated above.

The deposit was received August 26, 2003 by this International Depository Authority and has been accepted.

AT YOUR REQUEST: X We will inform you of requests for the strain for 30 years.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested September 4, 2003. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

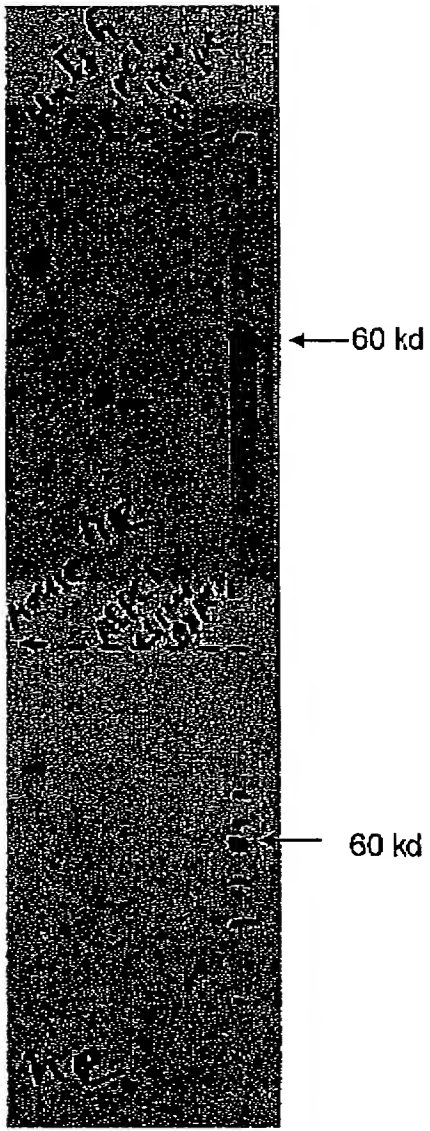
Marie Harris

Marie Harris, Patent Specialist, ATCC Patent Depository

Date: September 24, 2003

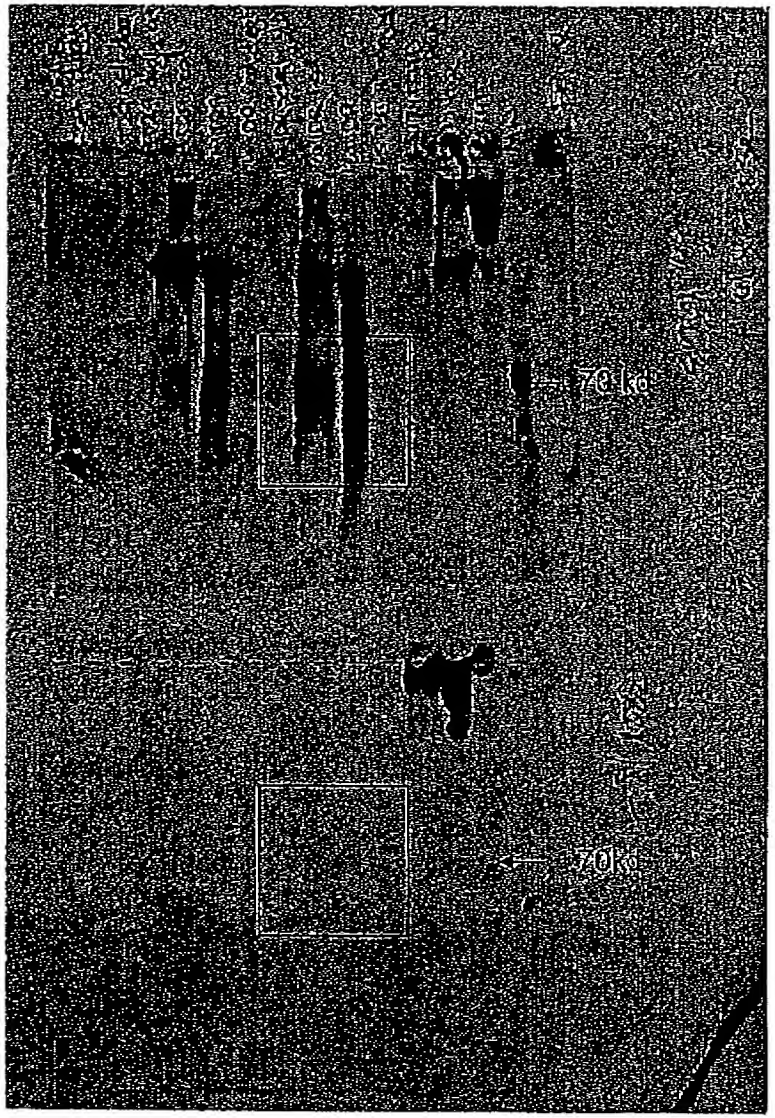
cc: Carmella L. Stephens
Ref: Docket or Case No.: A33081 072771.0106

Fig 1 Exhib 4



Comparison of murine mab31.1 and chimeric mab31.1.
Chimeric mab31.1 (top western) binds to a protein of approximately 40 kd as seen in the wells for aspc1 and ls174t. Although the intensity is Not as great, murine mab31.1 (bottom western) binds to the exact same Molecular weight protein for these same two tumor cell lysates. The size of the protein that these two mabs bind to is approximately 40 kd.

Fig 2 – Exhib 5



Western analysis of mab 33.28.
Two clones of mab 33.28 are compared. The bottom western
Uses mab 33.28 deposited at ATCC. Although the top western
Clearly demostrates greater binding to an antigen at the size
Described in the patent, eg 60-70 kd, the bottom western
Clearly shows a similar banding pattern albeit with less intensity.
I have boxed the region of the westerns compared for tumor
Cell lysate WIDR.